

CARNIVOROUS PLANT NEWSLETTER

Journal of the International Carnivorous Plant Society

Volume 42, No. 2

June 2013

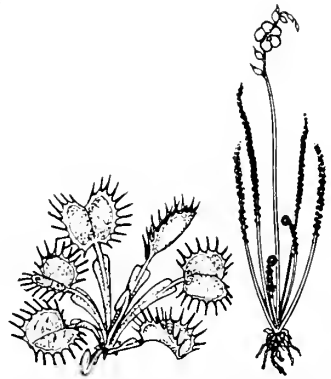




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Carnivorous Plant Society
www.carnivorousplants.org

Volume 42, Number 2
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Front Cover: *Pinguicula* × *gresivaudanica* growing in the French Alps. Photo by Aymeric Rocchia. Article on page 36.

Back Cover: *Aldrovanda vesiculosa* var. *rubescens* (A. Cross & L. Adamec) from south-west Western Australia, displaying a gradient of coloration due to variable sun exposure. All individuals originate from the same maternal plant, but have been independently grown from left to right in full sun, 50%, 70%, and 90% shade. Photo by Adam Cross. Article on page 57.

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Date of effective publication of the March 2013 issue of Carnivorous Plant Newsletter: 25 February 2013.

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Carnivorous Plant Newsletter is published quarterly in March, June, September, and December by the ICPS, Inc., 2530 Patra Drive, Richmond, CA 94803, USA. Periodicals postage paid at Richmond, CA and additional mailing offices. Postmaster: Send address changes to ICPS, Inc., PMB 322, 1564-A Fitzgerald Drive, Pinole, CA 94564-2229, USA. Printed by Allen Press, Inc., 810 E. 10th Street, Lawrence, KS 66044. Logo and masthead art: Paul Milauskas.

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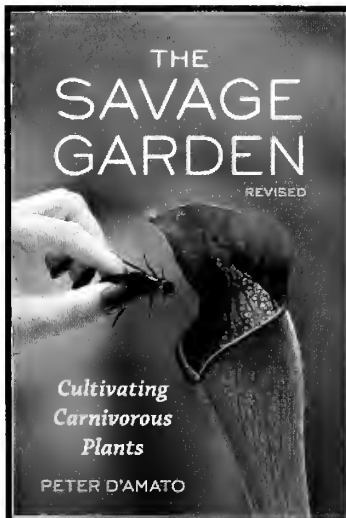
FAREWELL ERIC GREEN

Eric Green is probably familiar to most people interested in South African carnivorous plants. Not only did he discover many new *Drosera* taxa, which were later named by scientists from the late 1970s on, he also explored the Western Cape for new carnivorous plant habitats, and kindly shared his knowledge with carnivorous plant enthusiasts. Eric was a very skilled and passionate carnivorous plant grower, who had an amazing collection of South African *Drosera* (many of which he shared and traded with friends all over the world). He grew all kinds of carnivorous plants well; including *Heliamphora*, highland *Nepenthes*, *Sarracenia*, and *Pinguicula* in his greenhouses in Southfield, South Africa.

Sadly Eric passed away on Friday, 15 March 2013. He is survived by a son and daughter. He will be greatly missed by many carnivorous plant enthusiasts around the world. Through his generosity, the knowledge of South African carnivorous plants has been considerably increased, and plants that he shared now live on in collections around the globe. Farewell Eric.

A memorial article about Eric will be in the September issue of CPN.

ANDREAS FLEISCHMANN • ROBERT GIBSON • FERNANDO RIVADAVIA



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Pinguicula × *Gresivaudanica* (Lentibulariaceae), A NEW BUTTERWORT HYBRID FROM THE FRENCH ALPS

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Keywords: field studies: taxa: *Pinguicula* × *gresivaudanica*, French Alps

Abstract: A new natural hybrid of *Pinguicula* is here reported from the French Alps. It proceeds from the natural crossing of *P. grandiflora* Lam. subsp. *rosea* (Mutel) Casper with *P. vulgaris* L. subsp. *vulgaris*. The newly recorded hybrid is named *Pinguicula* × *gresivaudanica* because of the region where it has been discovered. The characters distinguishing this hybrid from other taxa are described, together with figures, a distribution map and a table. Fertility and chromosome number were also studied, supporting the hybrid nature of this plant.

Introduction

The butterwort genus (*Pinguicula* L., Lentibulariaceae) is composed of approximately 100 species (Rodondi *et al.* 2010; Yıldırım *et al.* 2012). They occur in the Old World from Europe to Japan via Siberia. In America, they can be found in Alaska, Canada, the Rocky Mountains, the Andes, and Tierra del Fuego. A few species can also be found in southeastern USA, one in Himalaya, and two in northern Africa. But the centers of diversity of this genus are found in Mexico, Cuba, and around the Mediterranean Sea (Steiger 1998; Yıldırım *et al.* 2012). However, only two natural hybrids have been described so far: *Pinguicula* × *hybrida* Wettst. (Wettstein 1919) and *P. × scullyi* Druce (Druce 1922).

Pinguicula × *scullyi* was described in 1922 by Druce from Irish material. It is a hybrid between the large-flowered butterwort (*P. grandiflora* Lam. subsp. *grandiflora*) and the common butterwort (*P. vulgaris* L.) that has also been recorded from Andorra (Partrat 2001) and the Alps (pers. obs.). *Pinguicula vulgaris* is a circumboreal species, quite common in suitable habitats in Alaska, Canada, northern USA, Iceland, Europe, and Russia. *Pinguicula grandiflora* only occurs in western Europe, from northern Spain, France (Pyrénées, Massif Central, Jura, and the Alps) and Ireland. It is also known from two locations in Switzerland and has been introduced in Britain (Heslop-Harrison 2004). In addition, it has recently been discovered in Northern Italy (Compostella *et al.* 2010). This species shows a great morphological variation and several infraspecific taxa have been described: i) *P. grandiflora* Lam. subsp. *rosea* (Mutel) Casper (Casper 1966), the pink-flowered butterwort occurring in the Isère, Savoie, and Haute Savoie departments in France (Fig. 1), ii) *P. grandiflora* Lam. f. *pallida* (Gaud.) Casper (Gaudin 1828), a light-blue flowered form native to the Jura Mountains, and iii) *P. grandiflora* Lam. f. *chionopetra* Nelson (Nelson 1993), a white-flowered form originating from Ireland and the Pyrénées (pers. comm.). Although these infraspecific taxa are known to occur sympatrically with *P. vulgaris* subsp. *vulgaris* in certain locations, only natural hybrids with *P. grandiflora* subsp. *grandiflora* have been recorded so far.

During fieldtrips to observe *P. grandiflora* subsp. *rosea* in its habitat, some unusual butterworts with larger and bluish flowers were found always growing sympatrically. Evidence is here provided for the hybridogenic origin of this odd plant and it is described under the name *Pinguicula* × *gresivaudanica* Roccia, *hybrida nova*.

Vegetative and floral morphology and morphometry: morphological (shape and color of leaves, calyx lobes, spur, corolla, and stigma) and morphometric (leaf number, length and width, scape number and length, calyx lobe size, spur length, corolla length and opening angle, corolla lobe length and width) characters were analyzed in habitat for 15 individuals. Measurements are presented as mean value \pm standard deviation (Table 1).

Chromosome number: chromosomes were counted on plates prepared as described in Casper & Stimper (2007). Root tips were treated with 0.002 M 8-hydroxyquinoline for 1.5 h at 14°C and fixed in ethanol:acetic acid (3:1) for 4 h at 4°C. They were then hydrolyzed in 2 M HCl for 10 min at 60°C. Root tips were water rinsed and squashed on glass slides. Chromosomes were stained with carmine acetic acid (50 ml acetic acid, 50 ml water and 4 g carmin powder) and counted using a light microscope.

Results

Pinguicula \times *gresivaudanica* Roccia, *hybrida nova* *P. grandiflora* Lam. subsp. *rosea* (Mutel) Casper \times *P. vulgaris* L. subsp. *vulgaris*.

Differs from *Pinguicula grandiflora* subsp. *grandiflora* in having a lavender corolla, corolla lobes as long as wide, a spur curved downwards and slightly shorter than in *P. grandiflora* subsp. *grandiflora* (proportionally to the full corolla length), a throat macula rounded in shape, outer corolla tube surface whitish, and above all in being sterile.

Description: perennial rosette-forming herb with numerous fibrous roots, forming hibernacula during winter. Summer leaves 5-7, obovate-oblong, obtuse, 6-8 \times 2-3.5 cm, flat on the ground, with entire and slightly involute margins, the upper surface covered with mucilaginous glands. Scapes (1-) 2-4 (-5), 10-18 cm in length, green, from sparsely glandular at the base to densely glandular just below the flower. Flowers (26-) 30-34 (-36) mm in length (spur included). Calyx pentamerous bilabiate, glandular; upper lip with 3 lobes, divided nearly to the base, obovate to triangular, acute to subobtuse, (3-) 3.5-4.5 (-5) mm in length; lower lip with 2 lobes, united from the base for (1/4-) 1/3 (-1/2) of their length, obovate to triangular, acute to subobtuse, 3.5-4.5 mm in length. Corolla bilabiate, lavender to Persian blue (see Table 1 for color), (26-) 30-34 (-36) mm in length; upper lip with 2 lobes, suborbicular, approximately as long as wide, 6-8 \times 6-8 mm; lower lip with 3 lobes, somewhat wavy and slightly overlapping, suborbicular, nearly as wide as long, the lateral ones 8-10 \times 8-10 mm, the median lobe bigger, 10-12 \times 10-12 mm; palate hairy, bearing a large white rounded spot, with violet veins; throat densely hairy, white with 2 dark spots at the base; spur approximately 3/10 of the total corolla length, (7-) 8-11 (-11.5) mm in length, darker than the corolla (columbine), cylindrical, curved downwards. Capsule never observed (drying before maturation). Seeds never observed (presumed sterile). Flowering from the second half of May to the first half of June. (Fig. 2 and 3)

Holotype: Crêt de Chazay (Les Adrets, Isère, France), 26 May 2012, Roccia, *MHNGr.2012.35958* (GRM)

Paratype: Tourbière de la Grande Côte (Chapareillan, Isère, France), 28 May 2012, Roccia, *MHNGr.2012.35960* (GRM)

Etymology: the epithet “*gresivaudanica*” refers to the Grésivaudan valley along which the hybrid has been discovered.

Chromosome number: $2n = \sim 40-50$

Table 1. Comparative vegetative and floral morphometry between *Pinguicula* × *gresivaudanica*, *Pinguicula grandiflora* subsp. *rosea*, *Pinguicula vulgaris* subsp. *vulgaris*, *Pinguicula* × *scullyi*, and *Pinguicula grandiflora* subsp. *grandiflora*. All observations and measurements were made at anthesis. Values are expressed as (mean value) ± (standard deviation) of 15 measurements (when possible). Due to grazing damage, it was not possible to complete measures for *Pinguicula* × *gresivaudanica* from the Tourbière du Bois de la Belle Aiguette.

Taxon	<i>Pinguicula</i> x <i>gresivaudanica</i>	<i>Pinguicula grandiflora</i> subsp. <i>rosea</i>	<i>Pinguicula vulgaris</i> subsp. <i>vulgaris</i>	<i>Pinguicula</i> x <i>scullyi</i>	Tourbière du Bois de la Belle Aiguette		Tourbière de la Grande Côte		
Station	Hydrotic location (Crêt de Chazay)		Tourbière du Bois de la Belle Aiguette						
Number of flowering plants	19	6	1	50+	50+	6	1	4	123
Growth type	temperate	temperate	temperate	temperate	temperate	temperate	temperate	temperate	temperate
Rosette type	homophyllous	homophyllous	homophyllous	homophyllous	homophyllous	homophyllous	homophyllous	homophyllous	homophyllous
Number	6.4 ± 0.8	5.5 ± 0.5	8.0 ± 0.0	4.9 ± 0.8	5.0 ± 0.0	7.0 ± 0.0	5.3 ± 0.6	5.0 ± 0.7	5.7 ± 0.9
Shape	obovate-oblong	obovate-oblong	obovate-oblong	obovate-oblong	obovate-oblong	obovate-oblong	obovate-oblong	obovate-oblong	obovate-oblong
Length (mm)	73.5 ± 6.1	46.5 ± 4.5	37.3 ± 0.6	33.3 ± 5.8	41.9 ± 3.7	63.0 ± 5.7	64.0 ± 6.3	36.5 ± 3.3	41.6 ± 11.6
Width (mm)	31.2 ± 4.5	24.7 ± 5.3	15.3 ± 1.5	17.3 ± 2.9	15.5 ± 2.7	23.6 ± 2.1	24.3 ± 3.3	15.9 ± 2.7	15.4 ± 2.2
Number	3.9 ± 1.2	2.0 ± 1.2	2.0 ± 0.0	1.7 ± 0.9	1.5 ± 0.6	10 ± 0.0	6.0 ± 0.0	2.3 ± 1.3	1.8 ± 0.3
Size (mm)	157.0 ± 23.3	106.4 ± 17.9		76.7 ± 10.4	92.5 ± 14.5	102.8 ± 9.4	155.7 ± 11.0	35.9 ± 12.6	103.8 ± 18.8
Upper lobe shape	obovate to triangular, acute, the apex of the medium one often divided in 2	obovate, obtuse		obovate, obtuse	obovate to triangular, obtuse	obovate	triangular, the apex of the medium one often divided in 2	obovate, acute	triangular, acute
Upper lobe size (mm)	3.7 ± 0.4	3.2 ± 0.6		3.1 ± 0.4	2.8 ± 0.2	2.8 ± 0.3	4.8 ± 0.6	3.6 ± 0.4	3.7 ± 0.6
Bottom lobe shape	obovate to triangular, acute, united at the base on 1/3 to 1/2	obovate, obtuse, united at the base on 2/3 to 3/4		obovate, obtuse, united at the base on 3/4	obovate, obtuse, united at the base on 1/2 to 1/3	obovate, united at the base on 1/2 to 1/3	triangular, united at the base on 2/5	obovate to triangular, acute, united at the base on 1/4	triangular, acute, divided to the base to united at the base on 1/3
Bottom lobe size (mm)	4.3 ± 0.5	3.4 ± 0.5		3.8 ± 0.5	3.5 ± 0.4	3.5 ± 0.4	4.8 ± 0.3	4.2 ± 0.6	3.2 ± 0.4
Color and shape	columnine, curved downwards	amarant purple, slightly curved downwards		amarant purple, slightly curved downwards	dark violet, curved downwards	columnine, straglit	dark violet, slightly curved downwards	columnine, curved downwards	dark violet, curved downwards
Length (mm)	10.8 ± 0.8	10.1 ± 1.1		8.6 ± 0.6	3.8 ± 0.8	7.5 ± 0.5	11.5 ± 0.5	8.4 ± 1.5	5.0 ± 0.7
Length, spur included (mm)	34.1 ± 2.2	32.0 ± 2.5		27.4 ± 2.6	17.4 ± 1.5	25.5 ± 0.7	33.0 ± 1.7	29.8 ± 3.7	23.0 ± 2.1
Color	light lavender	snow		snow	dark violet	dark violet	dark violet	perian blue	dark violet
Lower lip lobe	margins not or a little wavy, lobes not to slightly overlapping	margins wavy, lobes overlapping		margins wavy, lobes overlapping	margins not wavy, lobes not overlapping, divergent	margins a little wavy, lobes overlapping	margins not or a little wavy, lobes not to slightly overlapping	margins wavy, lobes overlapping	margins not wavy, lobes not overlapping, divergent
Throat	white rounded spot with white streaks	greenish rounded spot with not pink stripes		greenish rounded spot with not pink stripes	white rounded spot with white streaks	white rounded spot with white streaks	white rectangular spot with white streaks	greenish rounded spot with not pink stripes	with a white rounded macula on the palate, sometimes wearing a yellow spot

Table 1 Continued.

Opening angle (°)	82.0 ± 15.4	105.0 ± 13.1		105.3 ± 12.9	62.3 ± 14.4	90 ± 7.1	110.0 ± 18.0	111 ± 10.2	110.4 ± 13.4	64.3 ± 8.2
Upper lobe length (mm)	7.6 ± 1.1	6.6 ± 0.9		5.9 ± 0.6	3.5 ± 0.6	5.1 ± 0.2	8.0 ± 0.0	7.1 ± 1.1	5.7 ± 0.8	5.3 ± 0.6
Upper lobe width (mm)	7.3 ± 0.8	9.3 ± 1.3		6.5 ± 0.7	3.9 ± 0.4	5.3 ± 0.6	7.3 ± 0.6	7.4 ± 1.1	6.7 ± 0.7	5.3 ± 0.7
Lateral lobe length (mm)	9.3 ± 1.3	7.6 ± 1.4		6.4 ± 0.5	4.4 ± 0.7	6.4 ± 0.4	8.5 ± 1.0	9.2 ± 1.0	6.2 ± 0.8	7.5 ± 0.7
Lateral lobe width (mm)	9.5 ± 1.1	10.4 ± 1.5		7.2 ± 0.8	4.1 ± 0.5	5.6 ± 0.4	7.5 ± 0.9	8.4 ± 1.1	7.3 ± 0.8	5.6 ± 0.7
Median lobe length (mm)	11.2 ± 1.0	9.5 ± 0.8		7.5 ± 0.5	4.9 ± 0.7	7.2 ± 0.5	10.3 ± 0.6	10.9 ± 1.3	7.4 ± 0.9	8.7 ± 1.0
Median lobe width (mm)	10.5 ± 1.3	11.9 ± 1.6	pink	9.0 ± 1.1	4.7 ± 0.5	8.5 ± 0.3	8.8 ± 0.3	10.4 ± 2.1	8.7 ± 1.1	6.5 ± 0.7
Stigma					light yellow	light yellow	light yellow	white	pink	light yellow

Distribution: *Pinguicula* × *gresivandana* occurs in the Chartreuse and Belledonne mountain ranges where *P. grandiflora* subsp. *rosea* and *P. vulgaris* subsp. *vulgaris* can also be found (Fig. 1). The hybrid has only been found so far in three localities. The Haute-Savoie department is also home to *P. grandiflora* subsp. *rosea* and *P. vulgaris*. Their hybrid may also be present there.

Habitat and ecology: *Pinguicula* × *gresivandana* grows on calcareous seepages covered by mosses and in alkaline bogs. However, it is always found in shady places, well covered by trees, which is not a suitable lightning environment for most European butterworts (Fig. 2D).

At the type location, *P. × gresivandana* and *P. grandiflora* subsp. *rosea* grow in Hypnaceae mosses. This seepage is located at the edge of a spruce plantation and the butterworts share their habitat with *Carex hostiana* DC. (Cyperaceae), *Aquilegia vulgaris* L. (Ranunculaceae), *Rubus* sp. (Rosaceae), *Geranium robertianum* L. subsp. *robertianum* (Geraniaceae), *Ligustrum vulgare* L. (Oleaceae), *Lonicera xylosteum* L. (Caprifoliaceae) and some seedlings of *Acer pseudoplatanus* L. (Aceraceae).

At the Tourbière du Bois de la Belle Aiguette bog, *P. × gresivandana* grows along a small stream under the shade of *Betula pendula* Roth (Betulaceae) and is competing with *Callitha palustris* L. (Ranunculaceae). *Pinguicula grandiflora* subsp. *rosea* and *P. vulgaris* subsp. *vulgaris* can be found along the same stream but in sunnier places along with *Carex davalliana* Sm., *C. nigra* (L.) Reichard (Cyperaceae), *Viola palustris* L. (Violaceae) and *Saxifraga stellaris* L. (Saxifragaceae) among others. *Pinguicula grandiflora* subsp. *grandiflora* and *P. × scullyi* are growing in higher parts of the bog where *Sphagnum* species (Sphagnaceae) begin to colonize the peat surface. However, grazing has greatly reduced the numbers of plants of *P. × gresivandana* and *P. grandiflora* subsp. *grandiflora*, resulting in incomplete measurements in Table 1 for both taxa.

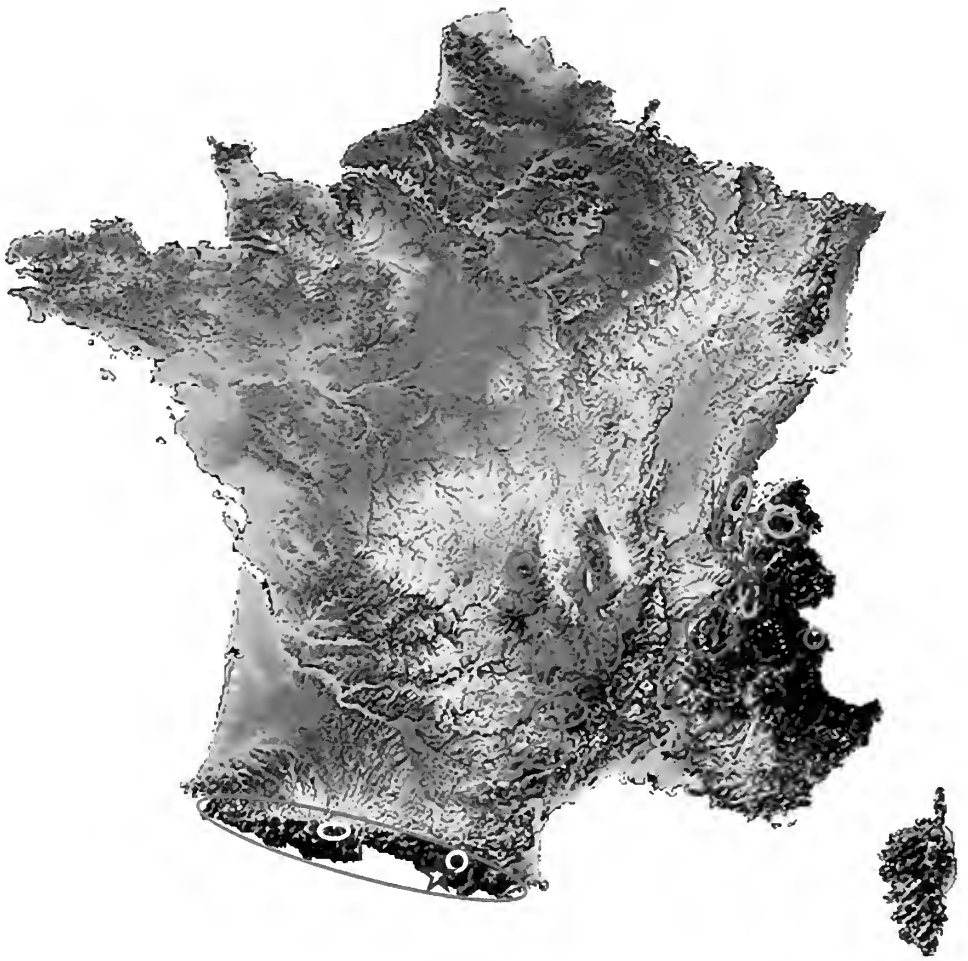


Figure 1: Distribution of *Pinguicula grandiflora* subsp. *grandiflora* (blue circles), *P. grandiflora* subsp. *rosea* (pink circles), *P. grandiflora* f. *pallida* (light blue circle), *P. grandiflora* f. *chionopetra* (white circles), *P. × scullyi* (violet stars), and *P. × gresivaudanica* (red star).

At the Tourbière de la Grande Côte bog, *P. × gresivaudanica* grows along a little stream among *Carex davalliana* Sm. (Cyperaceae) and *Equisetum* sp. (Equisetaceae), under pines and some willows and *Amelanchier ovalis* Medik (Rosaceae). *Pinguicula grandiflora* subsp. *rosea* and *P. vulgaris* subsp. *vulgaris* can be found in sunnier places, in a *Caricion davallianae* bog, sometimes dominated by *Phragmites australis* (Cav.) Steud. (Poaceae). *Pinguicula alpina* L. is also found growing near this site.

Relationship: The hybrid is morphologically close to *P. × scullyi* and *P. grandiflora* subsp. *grandiflora*. Both hybrids have long been mistaken for *P. grandiflora* subsp. *grandiflora*, explaining why *P. × gresivaudanica* remained unknown even if its localities were well botanized.

The hybrids of the two subspecies of *P. grandiflora* with *P. vulgaris* can be distinguished at first sight from their respective parent species by a corolla angle that is intermediate between both

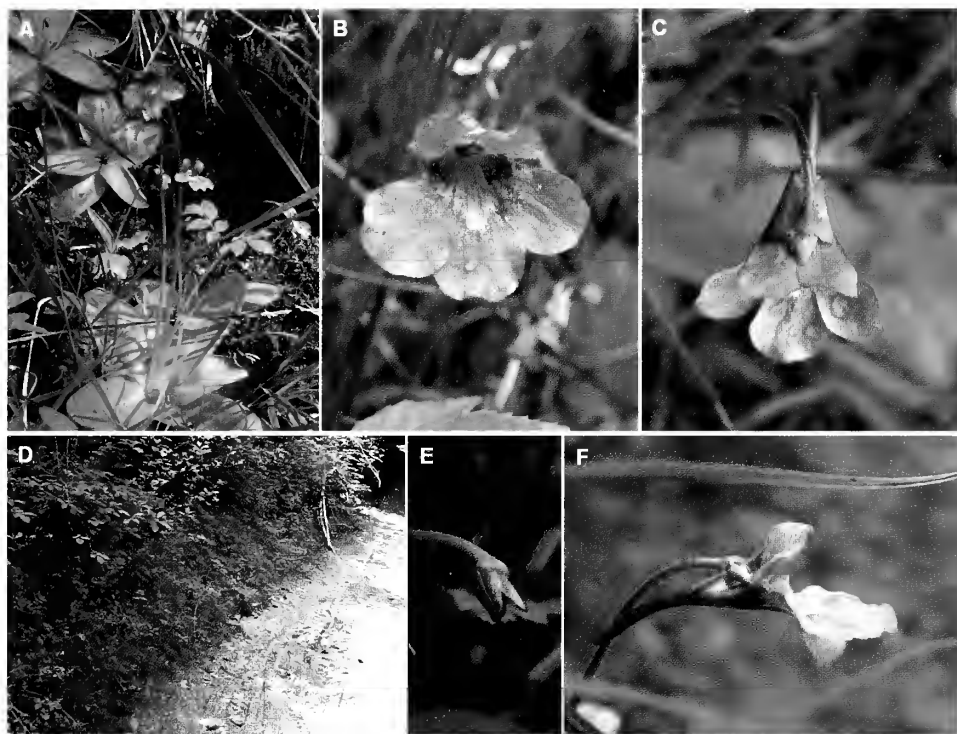


Figure 2: *Pinguicula* \times *gresivaudanica*. A: habit; B: front view of the flower; C: view from above; D: habitat; E: fruit at maximum development; F: lateral view of the flower. All pictures were taken at the type location.

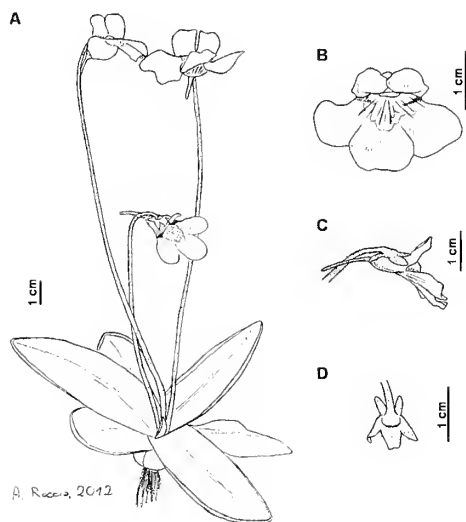


Figure 3: *Pinguicula* \times *gresivaudanica* from the type location. A: habit; B: front view of the flower; C: lateral view of the flower; D: calyx.

parents (Fig. 4 and Table 1). They bear a well-defined palate macula similar to the one of *P. grandiflora* subsp. *grandiflora* but it is rounded in the hybrids and rectangular in this subspecies. It is noteworthy that *P. \times gresivaudanica* has corolla lobes that are constantly as long as wide whereas they are constantly wider than long in *P. grandiflora* subsp. *rosea* and longer than wide for the lower lip lobes in *P. grandiflora* subsp. *grandiflora* and *P. vulgaris* subsp. *vulgaris*. The spur of *P. \times gresivaudanica* is also more eurved downwards than in *P. grandiflora*, a character reminiscent of the spur found in *P. vulgaris*. *Pinguicula* \times *scullyi* has flowers of a dark violet such as those of *Pinguicula vulgaris* subsp. *vulgaris* whereas *P. \times gresivaudanica* has a lavender to Persian blue corolla, quite intermediate in color between *P. grandiflora* subsp. *rosea* and *P. vulgaris* subsp. *vulgaris*. The

outer side of the corolla tube has the same color as the corolla lobes and spur in *P. × scullyi* whereas it is whitish in *P. × gresivaudanica*.

At its type location, *P. × gresivaudanica* grows along with a very few pale *P. grandiflora* subsp. *rosea* that tend to disappear because of the lack of luminosity in this shaded place. At the Tourbière du Bois de la Belle Aiguette, a single plant of the hybrid was found growing behind a small birch under a pine, where it does not receive any direct sunlight. Only 50 cm away, in direct sunlight, one can find *P. grandiflora* subsp. *rosea* and *P. vulgaris* subsp. *vulgaris*, both parents of this hybrid. Nearby in this same bog, *P. vulgaris* subsp. *vulgaris* grows sympatrically with *P. grandiflora* subsp. *grandiflora* and there, some *P. × scullyi* can be found. At its only known site in the Chartreuse massif, namely Tourbière de la Grande Côte, *P. × gresivaudanica* grows in absence of any of the putative parent species, but *P. grandiflora* subsp. *rosea* occurs in a bog at 20 m distance, and *P. vulgaris* subsp. *vulgaris* at 120 m distance. Ten years ago, *P. grandiflora* subsp. *rosea* was known to be much more widespread than today in this site (pers. comm.). It is possible that the hybrid once grew among the parental species and that rock movements and closure of the biotope led to the separation of the three taxa in this habitat of continuously moving screes with rapid movements.

The presence of *P. grandiflora* subsp. *rosea* growing sympatrically with or very close to *P. × gresivaudanica* at the three known locations of the hybrid, supports the assumed parentage. The second assumed parental species, *P. vulgaris* subsp. *vulgaris* is found growing sympatrically with or close to the hybrid in two of its three known sites. It is important to note that both *P. grandiflora* subsp. *rosea* and *P. vulgaris* subsp. *vulgaris* flower at the same period in their common locations. It is very unlikely that *P. grandiflora* subsp. *grandiflora* is one of the parents of this hybrid instead of *P. grandiflora* subsp. *rosea*, as the former is not known to grow at two of the three hybrid locations whereas in the last site it grows further distant. It is noteworthy that *P. × scullyi* is also known to

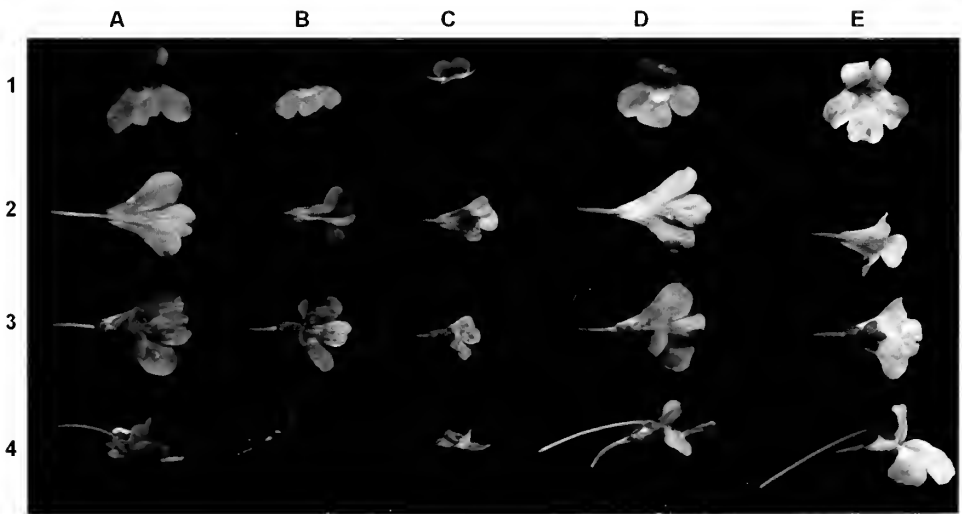


Figure 4: Comparative photographs of the studied species. Row A: *Pinguicula grandiflora* subsp. *grandiflora*; B: *Pinguicula × scullyi*; C: *Pinguicula vulgaris* subsp. *vulgaris*; D: *Pinguicula × gresivaudanica*; E: *Pinguicula grandiflora* subsp. *rosea*. Line 1: frontal view of the flower; 2: view from below; 3: view from above; 4 lateral view. All specimens are from the Tourbière du Bois de la Belle Aiguette, except *Pinguicula × gresivaudanica* that is from its type location. Photographs were taken according to Steiger, 1976. All flowers used for this figure are deposited in the voucher MHNGr.2012.35966.

grow in this bog and can be distinguished from *P. x gresivaudanica* (see above, Fig. 4 and Table 1). However, because *P. x gresivaudanica* occurs in shady places that are seemingly not suitable for either of the parental species, it is possible that new localities of the hybrid will be found where *P. grandiflora* subsp. *rosea* and *P. vulgaris* subsp. *vulgaris* would be absent, as it begins to happen in the site in the Chartreuse range.

Sterility: Flower visitors of this plant have been studied at the type location. Small insects belonging to the bee genus *Halictus* (Halictidae) (sweet bees) were observed entering and getting out of a flower to visit another in search of nectar or pollen (Fig. 5). However, returning to the site one month later, no seedpods could be found. It seems that ovaries of this plant soon desiccate after the corolla has been shed (Fig. 6). It is not a matter of environmental conditions as *P. grandiflora* subsp. *rosea* of this site do produce mature seedpods and seeds. During this study, it was also observed that *P. x scullyi* does not produce mature seedpods nor seeds while all other taxa, except *P. x gresivaudanica*, at the same location do make seeds.

Cultivation specimens of the plant studied in this article were also observed for this character. Flowers were hand-pollinated but only two mature capsules out of six pollinated flowers were obtained. However, those seedpods did not contain any seeds. So it is very likely that this plant is totally sterile, not a single seed grain could be observed, neither in *in situ* specimens nor in *ex situ* hand-pollinated plants. The infertility of a hybrid between *Pinguicula grandiflora* and *Pinguicula vulgaris* was predictable as both parents have different chromosome numbers, leading in a triploid sterile hybrid.

Although *P. x gresivaudanica* cannot reproduce in a sexual way, it produces many plantlets by gemmae formation around the winter hibernacula. This production is even greater than in *P. gran-*



Figure 5: Flower visitor of *Pinguicula x gresivaudanica*. A: the *Halictus* sp. on the flower lower lip after landing; B: the *Halictus* sp. visiting the corolla tube. Pictures were taken at the type location.

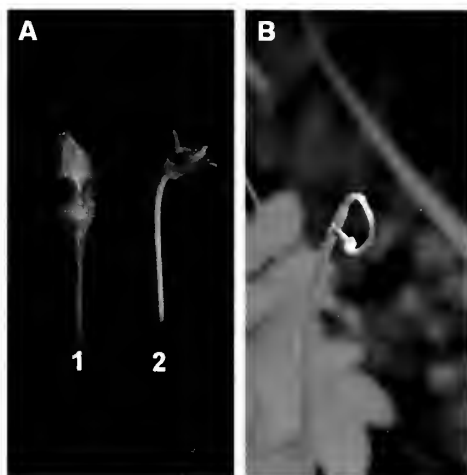


Figure 6: Different views of seedpods. A: Capsules at maximal development stage (1: *Pinguicula grandiflora* subsp. *rosea*; 2: *P. x gresivaudanica*), both have approximately the same age; B: typical drying *P. x gresivaudanica* seedpod. All plants are from the type location.

hybrid involving *P. grandiflora* subsp. *rosea* ($2n=32$ (Casper & Stimper 2007)) and *P. vulgaris* ($2n=64$ (Casper & Stimper 2007)) would be of $2n=48$. Thus, I assume that the real chromosome number of this plant is 48. This important fact supports the hybrid origin of *P. x gresivaudanica*.

Conservation: The total number of flowering plants of *P. x gresivaudanica* observed in its three known locations do not exceed 50 individuals. Thus, this taxon can be considered as "Critically Endangered" (CR) D following IUCN criteria. Moreover, as it only reproduces vegetatively, it cannot pretend to colonize new habitat as easily as its parents. Logging activities may represent a real danger. As *P. x gresivaudanica* tends to grow in shade, clearings in its habitat may be damageable (even if a single plant has been found in semi-shaded area in the Chartreuse site so that this taxon may survive in not so degraded conditions). In addition, grazing pressure has also shown to be damageable for *P. x gresivaudanica* and thus may be limited and/or plants protected from grazing.

Conclusion

The presence of *P. grandiflora* subsp. *grandiflora* in northern French Prealps may be overestimated due to confusion with *P. x gresivaudanica*. This hybrid is only known from three sites but its occurrence has not been studied in Haute Savoie where it is very likely to grow. Due to the very low number of flowering plants observed, it is considered as critically endangered following IUCN criteria.

Acknowledgements: The author would like to thank the Conservatoire Botanique National Alpin, the Parc Naturel Régional de Chartreuse and the Réserve Naturelle des Hauts de Chartreuse for permits to collect living material of protected taxa and on protected areas (arrêtés préfectoraux n° 2012-072-0045, DDT/SEEF n°2012-205 and n°2012-160-0024). The author also acknowledges R. Stimper for giving hints on kariotype preparation, and A. Fleischmann and F. Rivadavia for advice on this article. A. Fleischmann is also thanked for the flower visitor identification. Julie Delavie, cu-

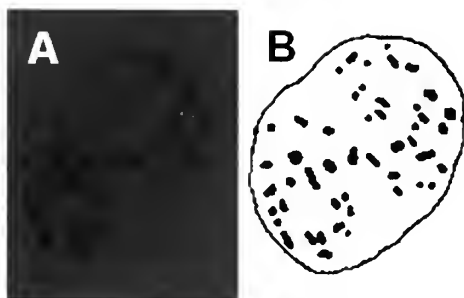


Figure 7: Karyotype of *Pinguicula x gresivaudanica* showing 48 chromosomes. A: light microscope photograph; B: interpretation drawing.

difflora and plantlets become adult in a much shorter time than for the species (pers. obs.).

Chromosome number: The chromosome number was difficult to obtain for *P. x gresivaudanica* due to the tiny size of the mitotic chromosomes, as in most butterwort species. However, most results gave a number between 40 and 50 (Fig. 7). The expected number for a

rator of the Muséum d'Histoires Naturelles de Grenoble herbarium, is also thanked for her advices on herbarium specimens.

Appendix

Other specimens examined:

- Pinguicula grandiflora* Lam. subsp. *grandiflora*; FRANCE. Tourbière du Bois de la Belle Aiguette (Saint Pierre d'Allevard, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35966* (GRM)
- Pinguicula grandiflora* Lam. subsp. *rosea* (Mutel) Casper: FRANCE. Crêt de Chazay (Les Adrets, Isère, France), 26 May 2012, *Roccia, MHNGr.2012.35959* (GRM); Tourbière de la Grande Côte (Chapareillan, Isère, France), 28 May 2012, *Roccia, MHNGr.2012.35961* (GRM); Tourbière du Bois de la Belle Aiguette (Saint Pierre d'Allevard, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35963* (GRM); Tourbière du Bois de la Belle Aiguette (Saint Pierre d'Allevard, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35966* (GRM).
- Pinguicula vulgaris* L. subsp. *vulgaris* L.: FRANCE. Tourbière de la Grande Côte (Chapareillan, Isère, France), 28 May 2012, *Roccia, MHNGr.2012.35962* (GRM); Tourbière du Bois de la Belle Aiguette (Saint Pierre d'Allevard, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35964* (GRM); Tourbière du Bois de la Belle Aiguette (Saint Pierre d'Allevard, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35966* (GRM).
- Pinguicula* × *gresivaudanica* Roccia: FRANCE. Crêt de Chazay (Les Adrets, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35966* (GRM)
- Pinguicula* × *scullyi* Druce: FRANCE. Tourbière du Bois de la Belle Aiguette (Saint Pierre d'Allevard, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35965* (GRM) ; Tourbière du Bois de la Belle Aiguette (Saint Pierre d'Allevard, Isère, France), 16 June 2012, *Roccia, MHNGr.2012.35966* (GRM)

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AGRICULTURAL WASTE MATERIALS AS COMPONENT OF ORGANIC POTTING
MEDIA FOR THE ENDANGERED *NEPENTHES TRUNCATA* MACF.
(PHILIPPINE PITCHER PLANT)

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Keywords: Charcoaled rice husk, coconut coir dust, coconut fiber, chemical and physical characterization, Sphagnum moss, *Nepenthes truncata*.

Abstract: Agricultural waste materials, namely, coconut fiber (CF), coir dust (CD), and charcoaled rice husk (CRH) taken singly or in combination with commonly used organic substrates wood charcoal chips (WCC), chopped tree fern (CTF), sphagnum moss (SM), and osmunda fiber (OF) were characterized for their suitability as components of mixed potting media for *Nepenthes truncata* (Macf.) plantlets. Several mixed media were evaluated, each with a component for aeration (WCC, CTF, or CRH), moisture retention (CF, CD or SM), and drainage (OF). Conventionally used container media mixes served as controls. Based on survival and leaf condition of plantlets, CD could replace SM, and both were superior to CF in moisture retention, while CRH was superior to wood charcoal chips and tree fern for aeration. Both CD and CRH are locally available and inexpensive compared to the materials they could replace. Small-sized plantlets performed better in Treatment 6 (T6) (2:1:1 mixture of SM, OF, and CRH) and Control I (pure SM) while medium-sized plantlets only preferred T6. Physico-chemical properties of T6 suggest that the appropriate medium for *N. truncata* plantlets is slightly acidic with low electrical conductivity, high porosity, moderate water holding capacity, and moderate water retention efficiency.

Introduction

Nepenthes truncata is an endemic plant of the Philippines, particularly in Mindanao (McPherson & Amoroso 2011). It is now considered as a commercially valuable ornamental but is classified as endangered by the IUCN (Clarke *et al.* 2006) due to slash and burn farming methods, over-collection from the wild, and poor cultivation practices of other crops such as vegetables and ornamentals. A suitable artificial media for cultivation of *N. truncata* will contribute to a technology package for propagated plantlets and reduce plant collection from the wild. All *Nepenthes* species naturally grow in low-nutrient habitats such as peat swamp, mountain rainforests, and pure sand (Horman 2000). In nurseries, soilless mixes composed purely of organic substances are commonly used as growing media (Greer 1998). In previous studies on cultivation of *Nepenthes* species, the growing media recommended were long fiber sphagnum moss (Butler 1990), a combination of osmunda fiber and sphagnum moss (De Kanel & Smith 1992), and perlite in combination with long fiber sphagnum moss and peat moss (Rice 2006).

Waste products are now being utilized as components in plant growing media. Among these, pruning waste compost (PWC) (Hernández-Apaolaza *et al.* 2005) and 7:3 peat-rice husk mixture (Marianthi 2006) have been used for pines, PWC plus spent mushroom compost (SMC) for orna-

mental plants (Benito *et al.* 2005), and compost from biosolids combined with yard trimmings for *Pachystachlys lutea* Nees (Wilson & Stofella 2000). Coconut fiber and coir dust have been used as substitutes for peat in many studies (Abad *et al.* 2002; Noguera *et al.* 2000; Wilson & Stofella 2000) due to properties similar to peat (Lennartson 1997). However, the suitability of coconut fiber or coir dust as growing media for epiphytic *Nepenthes* species has not yet been explored. Reuse of waste materials as alternative to other organic container substrates can help lessen the need for extracting resources from natural habitats, lessen residue accumulation in the environment (Raviv *et al.* 1986) and cut down the cost of growing media (Ingelmo *et al.* 1998). The study was conducted to test the suitability of agricultural waste materials (CF, CD, and CRH) in combination with locally available organic substrates (SM, OF, CTF, or WCC) for growing *N. truncata* in the nursery.

Materials and Methods

Physico-chemical characterization of media and their components. The mixed media as well as their components were characterized with three replicates per test. Determination of the water-holding capacity was done through a modification (use of hanging pot instead of a clay pot) of the procedure described by Holcomb (2004). For porosity (total and aeration) characteristics, a modification (use of hanging basket lined with plastic sheet instead of clay pot) of the procedure in the Nursery Production Factsheet by MAFF (2001) was followed. Water-retention efficiency of the potting mixes was determined by adopting the procedure of Nursery and Garden Industry Australia (2002). Electrical Conductivity (EC) and pH characterization was done by the Department of Agriculture, Davao City using 1:2 extraction method (Argo 2004).

Plant Material. Plantlets of *N. truncata*, collected from the wild and maintained for 3 months in a uniform medium of sphagnum moss at the Fuertes Nursery in Marilog District, Davao City, were used in the experiments (Fig. 1). Two arbitrary size categories were evaluated in the experi-



Figure 1: *Nepenthes truncata* mother plant (left) and representative plantlet used in the experiment (right).

Table 1. Composition of experimental organic potting media treatments for *Nepenthes truncata* cultivation.

Treatment	Components (% by volume)			
	Aeration (25)	Moisture retention (50)		Drainage (25)
	Experiments 1 & 2	Experiment 1	Experiment 2	Experiments 1 & 2
1	WCC	CF	CD	OF
2	CTF	CF	CD	OF
3	CRH	CF	CD	OF
4	WCC	SM	SM	OF
5	CTF	SM	SM	OF
6	CRH	SM	SM	OF

CF-coconut fiber; OF- osmunda fiber; WCC- wood charcoal chips; CTF- chopped tree fern root; CRH- charcoaled rice husk; SM- sphagnum moss

Table 2. Percentage of survival¹ of *Nepenthes truncata* Macf. plantlets in different potting mixes from January to July 2005.

Code	Potting mix	Mean % Survival of plantlets at specified interval (days) ²					
		30	60	90 ³	120	150	180
C1	Control 1	100	100	100a	100	100	100
C2	Control 2	100	90	90ab	90	90	90
C3	Control 3	100	100	90ab	80	70	50
C4	Control 4	90	80	70ab	60	50	40
T1	CF-OF-WCC	100	70	60b	30	10	10
T2	CF-OF-CTF	100	80	80ab	30	30	20
T3	CF-OF-CRH	100	100	100a	80	80	70
T4	SM-OF-WCC	100	100	90ab	80	80	80
T5	SM-OF-CTF	100	90	70ab	60	60	60
T6	SM-OF-CRH	100	100	100a	100	100	100
GRAND MEAN		99	91	85	71	67	62
P value (potting mix × plant size)		0.447	0.070	0.016	0.090	0.216	0.113

¹ data were transformed using square-root transformation

² means within columns without letters are not significantly different at alpha=0.05

³ means with common letter(s) are not significantly different using DMRT at alpha=0.05.

Control 1: 100% long fiber sphagnum moss;

Control 2: 50% peat moss, 25% perlite, and 25% pine tree bark;

Control 3: 33.33% river pebbles, 33.33% tree fern root, and 33.33% wood charcoal;

Control 4: 66.67% sphagnum moss, and 33.33% tree fern root

CF: 50% coconut fiber; OF-25% osmunda fiber; WCC- 25% wood charcoal chips; CTF- 25% chopped tree fern root; CRH-25% charcoaled rice husk; SM- 50% sphagnum moss

Table 3. Comparison of percentage of survival¹ of small and medium *Nepenthes truncata* Macf. plantlets in different potting mixes from January to July 2005.

Plantlet size	Potting mix	Code	% Survival of plantlets at specified interval (days) ²					
			30	60	90 ³	120 ³	150 ³	180 ³
Small	Control 1	C1	100	100	100a	100a	100a	100a
	Control 2	C2	100	80	80ab	80ab	80ab	80a
	Control 3	C3	100	100	100a	80ab	80ab	60ab
	Control 4	C4	80	60	40bc	40bc	40bc	20bc
	CF-OF-WCC	T1	100	40	20c	0c	0c	0c
	CF-OF-CTF	T2	100	60	60abc	0c	0c	0c
	CF-OF-CRH	T3	100	100	100a	100a	100a	100a
	SM-OF-WCC	T4	100	100	80ab	60ab	60ab	60ab
	SM-OF-CTF	T5	100	80	60abc	60ab	60ab	60ab
	SM-OF-CRH	T6	100	100	100a	100a	100a	100a
Mean			98	82	74	62	62	58
P value (across potting mixes)			0.456	0.086	0.015	0.000	0.000	0.000
Medium	Control 1	C1	100	100	100	100	100a	100a
	Control 2	C2	100	100	100	100	100a	100a
	Control 3	C3	100	100	80	80	60ab	40ab
	Control 4	C4	100	100	100	80	60ab	60ab
	CF-OF-WCC	T1	100	100	100	60	20b	20b
	CF-OF-CTF	T2	100	100	100	60	60ab	40ab
	CF-OF-CRH	T3	100	100	100	60	60ab	40ab
	SM-OF-WCC	T4	100	100	100	100	100a	100a
	SM-OF-CTF	T5	100	100	80	60	60ab	60ab
	SM-OF-CRH	T6	100	100	100	100	100a	100a
Mean			100	100	96	80	72	66
P value (across potting mixes)			No Value (subsets cannot be computed with alpha=0.05)		0.543	377	0.048	0.010

¹ data were transformed using square-root transformation

² means within columns without letters are not significantly different at alpha=0.05

³ means within columns with common letter(s) are not significantly different using DMRT at alpha=0.05.

Control 1: 100% long fiber sphagnum moss;

Control 2: 50% peat moss, 25% perlite, and 25% pine tree bark;

Control 3: 33.33% river pebbles, 33.33% tree fern root, and 33.33% wood charcoal;

Control 4: 66.67% sphagnum moss, and 33.33% tree fern root

CF: 50% coconut fiber; OF-25% osmunda fiber; WCC- 25% wood charcoal chips; CTF- 25% chopped tree fern root; CRH-25% charcoaled rice husk; SM- 50% sphagnum moss

ments: small and medium. Small plantlets have internode length (IL) ≤ 1 cm with at least 6 nodes while medium plantlets have either IL < 1 cm with at least 9 nodes or with IL > 1 cm with at least 4 nodes.

Experimental conditions for evaluation of nursery media. The experiments were conducted at a nursery in Marilog District, Davao City with a site elevation of 1200 m and a temperature range of 18 to 27°C. Light intensity ranged from 2200 to 18480 lux (SPER SCIENTIFIC 840020 digital light meter). Experiment 1 was conducted during the dry season (January to July 2005) with relative humidity range of 50.2 to 84.6% (VMR Traceable® Hygrometer), and average monthly rainfall of 101.57 mm as measured by the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) in Lanang, Davao City. Experiment 2 was conducted during the wet season (August to December 2005) with relative humidity range of 60.8 to 85.6% and average monthly rainfall of 155.52 mm.

Fertilizer application and watering. All plantlets were sprayed monthly with commercial foliage orchid fertilizer (Manusol®), with an NPK ratio of 19:19:19 and Mg, Cu, Mn, Zn micronutrients. Each formulation contained two tablespoons (about 30 g) of the fertilizer dissolved in 48 L of rainwater to obtain a concentration that was only one-third of the ordinary dosage for orchids to prevent over fertilization of the plantlets. Watering was done daily except during rainy days. Either spring water or stored rainwater was used and application was done using a hand-held atomizer.

Pest and fungi control. All plantlets were sprayed monthly with insecticide and fungicide using commercial organophosphate insecticide, profenofos (Selecron®), a systemic fungicide, metalaxyl-m (Ridomil®), and a broad-spectrum fungicide, mancozeb (Dithane®). Individual solutions of the chemicals were initially prepared prior to preparation of the spray solution as follows: Insecticide solution was prepared by mixing 1 ml profenofos (0.5 g·L⁻¹ active ingredient) to 1 L rainwater; systemic fungicide solution consisted of 3.9 g metalaxyl-m (0.0098 g·L⁻¹ active ingredient) added to 1 L rainwater; and broad-spectrum fungicide solution consisted of 1 g mancozeb (1.6 g·L⁻¹ active ingredient) to 1 L of rainwater. All solutions were thoroughly mixed and transferred to individual plastic bottles. To prepare the spray solution, 500 ml of each chemical solution was mixed. A spreader sticker (Hoestick®) was added to the pesticide and fungicide mixture at a concentration of 0.53 ml L⁻¹, to allow even distribution of the chemicals on the plant's surface. The insecticide-fungicide-sticker mixture was applied to the plantlets using a plastic hand-held atomizer.

Experiment 1: Use of coconut fiber as organic medium component. There were separate setups for small and medium plantlets, each laid out in a Completely Randomized Design (CRD) with four controls (commercial potting mixes), six experimental treatments, and five replicates, with one plant per replicate. The control treatments were: (C1) long fiber SM (Catalani 2000); (C2) 2:1:1 mixture of peat moss, perlite, and pine tree bark (Sarracenia Northwest 2012; Bilderback 2002); (C3) 1:1:1 mixture of river pebbles, tree fern root, and WCC (Vogelpeol 1980; Malesiana Tropicals n.d.); and (C4) 2:1 mixture of SM and CTF tree fern root (Malesiana Tropicals n.d.). The compositions of the experimental potting media are shown in Table 1. Survival and condition of the plantlets were monitored monthly for five months.

Experiment 2: Use of coconut coir dust as organic medium component. Due to high mortality rates observed in potting mixes with CF in Experiment 1, CF was replaced with CD (Table 2), which has physico-chemical properties comparable to SM (Cresswell 2001), in Experiment 2. The same four controls used in Experiment 1 were also used in Experiment 2, thus the total number of treatments in a single-factor analysis was 10. To determine the contributions of various components to the plant response, the 6 experimental media were also analyzed as 2 × 2 × 3 factorial experiment, where the factors were plant size (small or medium), component for moisture-retention (CD

or SM), and component for aeration (WCC, CTF, or CRH), respectively. As in Experiment I, the component for drainage (OF) was held constant. Survival and condition of the plantlets were monitored monthly for five months. The condition of the leaves and pitchers were evaluated using a 1 to 5 rating scale where 1 is least and 5 is best.

Data Analyses. Statistical analyses were done using Analysis of Variance (ANOVA) of a CRD and mean separation was done using DMRT. Pearson's Correlation Analysis was used to test for correlation between physical properties of potting mixes. For the percentage survival, data was transformed using square-root transformation.

Results and Discussion

Experiment 1: Use of coconut fiber as organic medium component.

The experiment suffered from moisture stress due to lack of rain and ineffective manual watering. At 60 days, there was a marked reduction in survival in some treatments; and in 90 days, there were statistically significant differences. C1 and T6 gave the highest and most consistent survival rates while T1 gave the lowest (Table 2).

The survival rates of small plantlets were lower than that of medium plantlets (Table 3). Severe symptoms of water stress such as yellowing of leaves and curling of leaf blades accompanied low survival rates. No new shoots were produced and lower leaves tend to undergo abscission.

Plantlets grown in coconut fiber-based mixes have respectively lower survival rates of 46.7% ($p=0.007$), 40% ($p=0.001$), and 33.3% ($p=0.00$) for 120, 150, and 180 days after transfer compared to a consistent 80% survival rate for plantlets grown in media with sphagnum moss.

Experiment 2: Use of coconut coir dust as organic medium component

A 100% survival after 150 days of observation was obtained in all potting mixes in contrast to the varied survival rates of the plantlets in Experiment I. This is not only due to the use of CD instead of CF in the growing medium but also due to higher amount of rainfall when Experiment 2 was conducted. There was no significant difference among the controls (C1 to C4) in terms of leaf condition. However, among 6 experimental treatments, T1 with CD was inferior ($p=0.01$) compared to potting mixes T3 with CD, T5, and T6. Treatments T5 and T6 were also significantly better in leaf condition ($p=0.01$) than all 4 controls and the other experimental treatments. Of the three factors (plant size, component for moisture-retention, and component for retention) only plantlet size had a significant effect on the condition of *N. truncata* leaves. Throughout the experiment, the leaf condition of the small plantlets were significantly better than that of medium plantlets ($p=0.03$). Coir dust and sphagnum moss were comparable, while rice husk was better than wood charcoal and tree fern among the components for aeration.

Physico-chemical characterization of organic substrates and potting mixes in relation to plant growth. A growing medium with EC readings below the critical level of $2.0 \text{ mmhos}\cdot\text{cm}^{-1}$ is considered a good substrate for the plant (A & L Plains Agricultural Lab, Inc. 2001; Argo 2004; Harris Laboratories 2009). Other suitable physico-chemical properties include: pH between 6.5 and 7.0 but some species have developed adaptations that allow them to thrive at a higher (Mauseth 1998) or lower pH levels like *Nepenthes* (Polunin 1994), WHC within 0.60 to $0.80 \text{ g}\cdot\text{ml}^{-1}$ (Strain 1999), WRE values greater than 80% (NGIA 2002), AP of at least 20 to 25% and as high as 45% in warmer temperatures with TP that must be greater than 60 to 70% (MAFF 2001). Based on physico-chemical analyses, the EC indicate that all the potting substrates and potting mixes are suitable. Since *N. truncata* plantlets prefer a slightly acidic medium (Polunin 1994), the majority of the mixes are found suitable except T1 with CF, T4, and C3, which are basic. WHC and WRE were highly correlated and

Table 4. Physico-chemical properties of different potting media for plantlets of *Nepenthes truncata* Macf.

Potting Mixes	Code	Physico-Chemical Properties ¹					
		AP	TP	WHC	WRE	EC	pH
Control Treatments							
Control 1	C1	61.02a	75.23a	0.26f	25.70bcd	0.72	6.6
Control 2	C2	38.09a	59.17a	0.45c	16.70e	0.33	5.1
Control 3	C3	42.13a	60.39a	0.02h	20.00de	0.24	7.8
Control 4	C4	52.59a	63.94a	0.32d	1.33f	0.63	6.6
Experimental Treatments							
CF-OF-WCC	T1 with CF	52.79a	69.68a	0.06e	2.33e	0.51	8.4
CF-OF-CTF	T2 with CF	49.53a	63.46a	0.03e	4.33e	0.27	6.0
CF-OF-CRH	T3 with CF	43.26a	61.16a	0.17d	11.00d	1.15	5.3
CD-OF-WCC	T1 with CD	40.23bc	53.67b	0.46c	30.00ab	0.44	6.8
CD-OF-CTF	T2 with CD	42.23abc	48.92b	0.67a	28.30abc	0.32	6.6
CD-OF-CRH	T3 with CD	41.14bc	42.96b	0.63bc	32.30a	0.31	6.2
SM-OF-WCC	T4	60.03a	80.62a	0.21g	27.30abc	0.60	7.8
SM-OF-CTF	T5	52.16a	63.26a	0.28ef	16.00e	1.02	6.7
SM-OF-CRH	T6	61.58a	77.72a	0.29de	23.30cd	0.85	6.0
Mean		48.98	67.46	0.36	22.10	0.57	6.6
P value		0.03	0.03	0.00	0.00		
Coefficient of Correlation ²		0.00 ³		0.00 ⁴			

¹ means within columns with common letter(s) or no letters are not significantly different using DMRT at alpha=0.05.

² correlated at 0.05 level

³ correlation between AP and TP

⁴ correlation between WHC and WRE

AP: aeration porosity (%); TP- total porosity (%); WHC- water-holding capacity (g ml⁻¹); WRE- water-retention efficiency (%); EC- electrical conductivity (mmhos*cm⁻¹); Expt- Experiment

Control 1: 100% long fiber sphagnum moss;

Control 2: 50% peat moss, 25% perlite, and 25% pine tree bark;

Control 3: 33.33% river pebbles, 33.33% tree fern root, and 33.33% wood charcoal;

Control 4: 66.67% sphagnum moss, and 33.33% tree fern root

CF: 50% coconut fiber; OF-25% osmunda fiber; WCC- 25% wood charcoal chips; CTF- 25% chopped tree fern root; CRH-25% charcoaled rice husk; CD-50% coconut coir dust; SM- 50% sphagnum moss

only T2 with CD and T3 with CD fall within the required WHC range while the WRE for all mixes are less than 80% (unsuitable). For porosity characteristics, AP and TP were found to be highly correlated. AP readings for all mixes were above 20 to 25% (suitable) while TP readings of all mixes except C2 and T1 with CD, T2 with CD, and T3 with CD are greater than the standard 60%. Potting

Table 5. Physico-chemical properties of individual organic substrates for plantlets of *Nepenthes truncata* Macf.

Substrates	Water retention efficiency ¹ (%)	Electrical conductivity (mmhos cm ⁻¹)	pH
Coconut coir dust	35.67b	0.27	7.1
Coconut fiber	6.67de	0.69	7.1
Charcoaled rice husk	38.33b	1.01	5.7
Osmunda fiber	2.67e	1.43	6.4
Peat moss	25.67c	0.32	4.9
Perlite	86.67a	0.14	7.1
Pine bark chips	88.67a	0.59	5.2
River pebbles	2.67e	0.11	8.0
Sphagnum moss	38.33b	0.96	6.8
Tree fern root	1.00e	0.34	5.5
Wood charcoal	3.00e	0.52	8.0
Mean	29.94	0.58	6.53
P value	0.00		

¹ means within column with common letter(s) or no letters are not significantly different using DMRT at alpha=0.05.

mixes with coconut coir dust tend to hold more water compared to the other mixes (Table 4).

Among the components (Table 5), wood charcoal and river pebbles may be too basic and not ideal to be used while peat moss, tree fern, charcoaled rice husk, and pine bark chips are acidic and may be suitable. Thus, treatments with wood charcoal or river pebbles have lower rates of survival than most of those with acidic components in Experiment 1. In terms of WRE, pine bark chips and perlite are significantly better compared to other components (Table 5). Thus, C2 potting mix was favorable for the survival of the plantlets in Experiments 1 and 2.

It seems that the combination of acidic reaction and intermediate WRE from sphagnum moss and charcoaled rice husk make an ideal potting mix as shown by high survival rates of both small and medium plantlets of T6 (Tables 3, 4) in Experiment 1. Based on physico-chemical properties, coir dust may be used as a substitute to sphagnum moss. However, beneficial microorganisms found in sphagnum moss that prevents root rot pathogens (Premier Press 2000), probably absent in coconut coir dust, may help in the establishment of *N. truncata* plantlets.

Conclusions

The organic substrates coir dust (CD) and sphagnum moss (SM) could be interchangeable, and both were superior to coconut fiber (CF) as a component for moisture retention, while charcoaled rice husk (CRH) was superior over wood charcoal chips (WCC) and chopped tree fern (CTF) as a component for aeration based on survival and leaf condition parameters. Both small and medium plantlets performed better in Treatment 6 (T6) (2:1:1 mixture of SM, OF, and CRH) and C1 (SM). Leaf condition was found to be significantly better in Treatment 5 (2:1:1 mixture of SM, OF, and CTF) and T6 compared to other mixes while small plantlets have significantly better leaf condi-

tion than medium plantlets. Physico-chemical properties of the superior treatment, T6, suggest that an appropriate medium for growing *Nepenthes truncata* should have the following characteristics: slightly acidic (pH=6.8), moderate electrical conductivity (0.85 mmhos·cm⁻¹), high aeration porosity (61.58%) and total porosity (77.72%), and moderate water-holding capacity (0.29 g·mL⁻¹) and water retention efficiency (23.30%).

Acknowledgments: This study was supported by Philippine Council for Agriculture, Forestry, and Natural Resources Research and Development (PCCARD), University of the Philippines Research Grant and Department of Agriculture, Bureau of Plant Industry.

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TURION DEVELOPMENT IS AN ECOLOGICAL TRAIT IN ALL POPULATIONS
OF THE AQUATIC CARNIVOROUS PLANT
ALDROVANDA VESICULOSA (DROSERACEAE)

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Keywords: hibernacula, overwintering, vegetative dormancy.

Aldrovanda vesiculosa L. is a globally rare aquatic carnivorous plant, recently listed as Endangered by the International Union for the Conservation of Nature (IUCN) in light of significant population decline worldwide over the last century (Cross 2012a). Fewer than 50 extant natural populations are now believed to exist worldwide (Cross 2012b). However, despite the recent focus afforded to the species in the face of its decline, several aspects of its ecology and biology remain unclear. As part of a concerted global effort to conserve, restore, and increase awareness of the threats faced by *A. vesiculosa*, this paper discusses population differentiation in the context of coloration and the production of turions (hibernacula).

The expansive distribution of *A. vesiculosa* encompasses almost a dozen climatic zones from tropical monsoonal to subarctic (see Peel *et al.* 2007), and extends some 84° in latitude from the Limpopo River in southern Africa to Lake Ladoga in northern Russia (Fig. 1), and over 160° in longitude from eastern Ghana to Bundaberg on the eastern coast of Australia (Cross 2012b). This range represents one of the most expansive climatic tolerances known for any angiosperm species. In order to persist in such a wide variety of climates, *A. vesiculosa* must rely on one of two physiological characters: either 1) the possession of an extraordinarily broad ecological tolerance (plasticity) for abiotic factors such as temperature and seasonality, particularly in relation to the timing and success of reproduction and overwintering, or 2) the development of specific tolerances or adaptation to climatic and seasonal conditions at a regional or local scale. Both of these scenarios have differing implications for population genetics, dispersive and colonization capacity, and the ability of populations to respond to environmental alteration through human activity or climate change.

Habitat degradation and fragmentation are believed to be among the principal causes of decline in *A. vesiculosa* (Jennings & Rohr 2011), with suitable habitat becoming increasingly scarce and exacerbating the heavily disjunct nature of the species' distribution. Though *A. vesiculosa* appears to demonstrate a rather unprecedented genetic uniformity, with molecular studies presenting extremely low levels of variation between individuals from populations isolated by many thousands of kilometers (Elansary *et al.* 2010; Hoshi *et al.* 2006; Maldonado San Martín *et al.* 2003; Adamec & Tichy 1997), recent data supports a distinction between European and non-European accessions (Elansary *et al.* 2010; Maldonado San Martín *et al.* 2003). Research to further elucidate genetic variation in the species is ongoing, however such limited polymorphism strongly suggests that *A. vesiculosa* possesses an extraordinary adaptive capacity and broad ecological tolerance, rather than a number of discretely adapted populations.

Two morphological characters have traditionally been used to differentiate between the two inferred genetic clades: pigmentation (the presence or absence of an anthocyanin expression in plant tissues), and the production of turions (Maldonado San Martín *et al.* 2003; Breckpot 1997). Plants originating from central and southern Africa, Australia, and western Hungary display the

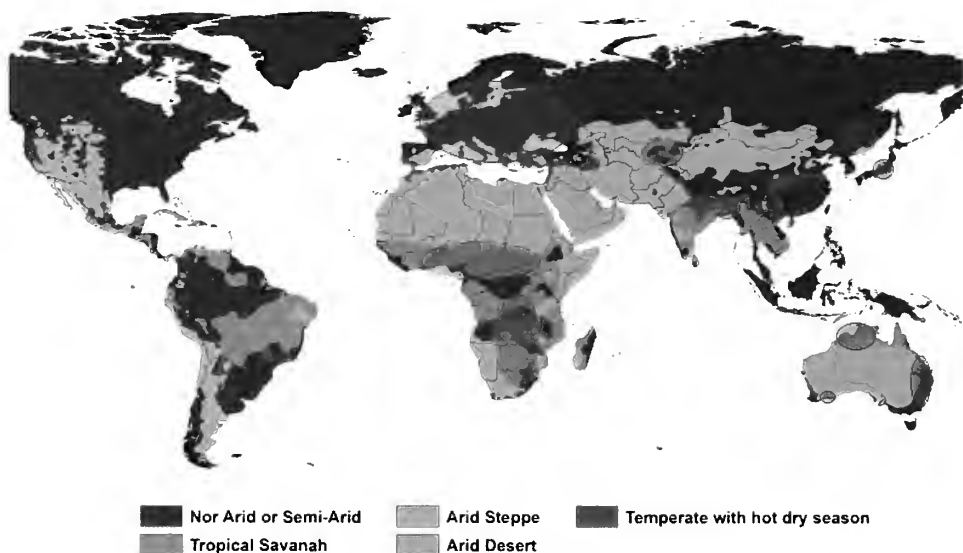


Figure 1: Map indicating Köppen climatic zones grouped by precipitation (from Achten *et al.* 2012) overlaid with ellipses showing the generalized historic population loci for *Aldrovanda vesiculosa*.

characteristic reddening associated with anthocyanin production (*A. vesiculosa* var. *rubescens*; Cross 2012b), while all other known extant populations lack this pigmentation (*A. vesiculosa* var. *vesiculosa*). Herbarium specimens from East Timor and northern equatorial Africa suggest that these populations may also have possessed anthocyanin; however the coloration of Hungarian plants is an exception to the European/non-European population distinction. As anthocyanin production is regulated by at least five associated enzymatic processes, the disruption of even a single pathway through environmental pressures resulting in the loss of pigmentation (Gould *et al.* 2008; Andersen & Markham 2006), it seems unlikely that coloration explains the inferred genetic difference (Back Cover). The sympatric occurrence of pigmented and non-pigmented populations within a relatively small geographic area in Eastern Europe also suggests that gene flow may be even lower than expected between *A. vesiculosa* populations.

The differentiation of populations based on the production of turions remains a matter of some debate. Some growers and researchers believe that populations of *A. vesiculosa* var. *rubescens* are not capable of producing overwintering structures, based on the observation that plants tend to slow or halt growth during the colder months. This may be perpetuated by suggestions that only non-pigmented Eurasian populations are capable of producing fully dormant hibernacula (Adamec 2005; Breckpot 1997), despite published evidence to the contrary (*e.g.* Adamec 1999a). Adamec (2005) notes that plants originating from the Okavango Delta in Botswana and from Lake Balata-to in Hungary, both pigmented, readily form dormant turions. Lájér (2006) confirms the production of winter buds by plants from Hungary. Zaman *et al.* (2011) also note the presence of turions on plants collected from Bangladesh. During an analysis of *A. vesiculosa* herbarium records from around the world by the author, turions were observed on specimens from France, Germany, Austria, Switzerland, Russia, Poland, Lithuania, Romania, Italy, and Japan (*A. vesiculosa* var. *vesiculosa*). These records are supported by numerous recent studies, with turion formation in various populations observed either at field sites or in cultivation (*e.g.* Adamec 2005, 2003, 1999b; Vilkonis 2004; Komiya 1989;



Figure 2: Turions forming in early winter (June) on *A. vesiculosa* individuals from Esperance, southwest Western Australia, cultivated at Kings Park and Botanic Garden (Perth, Western Australia).

Kamiński 1987a,b). Darnowski (2002) mentions the development of hibernacula on plants from the Northern Territory of Australia (*A. vesiculosa* var. *rubescens*), as does Adamec (1999a). Turions have been found in the sediment of high altitude *A. vesiculosa* var. *rubescens* habitat in Armidale, New South Wales (D. Bell, pers. comm.), and observed on cultivated plants from other coastal areas of eastern Australia (Adamec 2003a). Recently, turions have also been observed by the author to form readily on pigmented Australian plants originating from Esperance (southwest Western Australia; Fig. 2), Darwin (Northern Territory), Broulee (southeast New South Wales), and the Kimberley (northern Western Australia; Cross, unpubl.). The only regions remaining for which no record of turion production exist are those from East Asia (Kazakhstan, China, North and South Korea), Southeast Asia (East Timor), and equatorial Africa north of Botswana. *Aldrovanda vesiculosa* is poorly represented in herbarium records from all three regions, and there is little or no information available on the persistence of any of these populations in the last few decades.

It may be concluded therefore that every *A. vesiculosa* population, irrespective of geographical location or coloration, possesses the intrinsic potential to produce turions in response to unfavourable conditions. Failure to do so indicates that plants in any given region may not be experiencing the climatic cues to which they have become locally adapted in their location of origin, or that conditions never become adverse enough to require turion development (see Sculthorpe 1967). This variable response may result in the varying depths of dormancy presented in detail by Adamec (2003b), who notes that populations of tropical affinity produced only weakly dormant buds (termed imposed dormancy, in contrast to the innate dormancy described for populations experiencing regular and pronounced adverse winter conditions). This does not necessarily imply that populations from dif-

ferent climates are ecologically discrete, more that they are likely to possess complex and deeply ingrained autoecological mechanisms that may take several seasons to adapt to new conditions. While the depth of dormancy may be seen to vary temporally or spatially, the ability to produce overwintering structures remains an innate ecological feature.

For example, plants of several Australian (originating from Esperance, Darwin, Broulee, and the Kimberley) and one east Polish population are cultivated at Kings Park and Botanic Garden, Perth, Western Australia (experiencing a Mediterranean climate with warm, dry summers and cool, wet winters). During the relatively mild winter of 2011, where temperatures rarely fell below 8–10°C, turion formation was observed only on Polish individuals (~60%). These remained dormant on the substrate of the cultivation tank until mid-spring, contrasting with the vigorous growth of Australian plants in early spring when water temperatures rose to around 25°C. However, after several weeks of uncharacteristically cool weather in the winter of 2012 (minimum 2–6°C), widespread turion development was observed in all populations. The buds detached, sank, and remained dormant on the substrate uniformly until mid-late spring, when all of the populations began new season growth.

The evidence suggests that neither pigmentation nor turion production are likely to represent significant differentiating factors in *A. vesiculosa* population genetics, and highlights the necessity for genetic studies utilizing both high resolution and highly reproducible techniques. Perhaps as these studies progress we may find some answers to long-held questions regarding population structure, gene flow, and the origin of the species. However, in the face of its continuing decline, the ecological plasticity and apparent adaptive capacity of *A. vesiculosa* suggests a very heartening possibility: if the loss and degradation of the species' habitat is alleviated, remaining populations, however rare they may be, may well possess a significant capacity to slowly adapt to a changing environment if carefully safeguarded and preserved.

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NEPENTHES KHASIANA'S CHITIN-INDUCED PITCHER LIQUID: A POTENTIAL TREATMENT FOR OPPORTUNISTIC FUNGAL INFECTION

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Keywords: *Nepenthes khasiana*, fungal infection, antimycotics, naphthoquinone, secondary metabolite

The endangered tropical pitcher plant *Nepenthes khasiana* has evolved mechanisms to lure, capture, and digest prey animals (USDA 2003; Eilenberg *et al.* 2010). Native to areas with nutrient-poor soil, consuming insects enables *Nepenthes* to gain additional nourishment (Mithofer 2011). Located at the ends of leaf tendrils, their pitchers' bright colors and nectar secretions are a powerful attractant for bugs (Mithofer 2011). Venturing insects slip on the pitcher's waxy mouth and fall inside, ultimately drowning in the liquid (Rischer *et al.* 2002). Glands located at the base of the pitcher secrete hydrolyzing enzymes (Eilenberg *et al.* 2006). This serves to break the arthropod's body apart so it can be absorbed by the plant as sustenance (Eilenberg *et al.* 2006). Under special circumstances, *Nepenthes* pitcher liquid will also contain potent antifungal compounds called naphthoquinones (Eilenberg *et al.* 2010). Their purpose, it is believed, is to prevent captured prey from being consumed by fungi, a competing organism (Eilenberg *et al.* 2010). For hundreds of years, naphthoquinones have been utilized in Asia and South America for medicinal purposes (Babula *et al.* 2009). In the United States, emerging studies are investigating how these compounds can be produced, as well as their efficacy against opportunistic human fungal pathogens (Eilenberg *et al.* 2010). The importance of this research is underscored by the limited variety of antifungal drugs that are commercially available, as well as their susceptibility to pathogenic resistance and cross-tolerance (Kayser *et al.* 2003).

Naphthoquinones are secondary metabolites produced by *Nepenthes*' pitcher liquid (Rischer *et al.* 2002). Secondary metabolites are not essential for a plant's regular growth, but aid in lesser functions like defense against herbivory (Bio-Medicine 2012). For centuries, many cultures have been utilizing this type of organic compound for medicinal applications (Karuppusamy 2009). Antibiotics, widely used to treat bacterial infections, are secondary metabolites of mold (ePlant-science.com 2009). One major drawback of antibiotic medication is that its use can suppress the immune system by killing microbes which ward off infection (Merck 2008). Women taking antibiotics are at risk for developing candidiasis, which is named after its causal fungal strain *Candida albicans* (NIH 2010). Also known as vaginal yeast infection, candidiasis is responsible for more than 10 million doctor visits in the United States each year (Merck 2008). People with compromised immune systems, like the elderly and those with HIV/AIDs, are at risk for developing a more serious form of candidiasis (Merck 2008). This potentially fatal infection occurs when *C. albican* spores infiltrate the bloodstream (Merck 2008). There is a limited variety of commercial antimycotics (antifungal drugs) that are currently available (Eilenberg *et al.* 2010). Over-usage of these antimycotics, which tend to operate by the same mechanism of action, is creating drug-resistant fungal strains (Kayser *et al.* 2003). Moreover, concomitant use of antifungal drugs is causing cross-resistance in pathogens (Eilenberg *et al.* 2006). Developing antimycotics which function differently than current ones is needed to ensure our continuing ability to treat fungal infections.

Eilenberg *et al.* (2006) demonstrated a method of inducing the production of antifungal secondary metabolites known as naphthoquinones in *Nepenthes khasiana*. Published by the Journal of Experimental Botany, the article entitled "Isolation and characterization of chitinase genes from

pitchers of the carnivorous plant *Nepenthes khasiana*” showed that naphthoquinone production can be induced via colloidal chitin injections into closed *N. khasiana* pitchers. Chitin is the main component of insect exoskeleton, and is also an integral part of fungal cell walls (Collinge *et al.* 1993). Chitinase, an enzyme which degrades chitin, was found to be an implicit component of *N. khasiana* pitcher liquid. Chitinase appears to function in both carnivory, by breaking-down insects, and defense, by degrading fungi. The liquid of closed *N. khasiana* pitchers (referred to as “prey-challenged”) did not contain naphthoquinones, but two types of chitinases were present. When immature *Nepenthes* pitchers were injected with chitin, naphthoquinones and a third type of chitinase were detected. Chitinase was also found in open pitchers containing prey, but naphthoquinones were not. Though the pitcher liquid does not appear to contain the potent antifungal naphthoquinones under normal circumstances, it may still exhibit antifungal activities due to the endogenous presence of chitinase. This might explain why there is “anthropological evidence of [people] using the liquid from unopened traps as a curing means” (Eilenberg *et al.* 2010).

In 2010, the Journal of Experimental Botany published a compelling article entitled “Induced production of antifungal naphthoquinones in the pitchers of the carnivorous plant *Nepenthes khasiana*”. In this study, Eilenberg *et al.* (2010) examined the antifungal effects of naphthoquinones on various strains of human pathogenic fungi. Similar to previous experiments, the team induced naphthoquinone production by injecting closed *Nepenthes khasiana* pitchers with colloidal chitin. The *N. khasiana* pitcher liquid of chitin-induced, prey-challenged, and open pitchers containing prey was then screened for the presence of naphthoquinones. Only the chitin-induced pitcher liquid contained these secondary metabolites. The absence of naphthoquinones from open pitchers containing prey implies that chitin alone is not responsible for its production. However, there is evidence that the components of chitin are needed to form the naphthoquinone compound (Rischer *et al.* 2002). Eilenberg *et al.* postulate that injecting the pitchers with a syringe simulates a predatory presence. As previously discussed, the major natural role of secondary metabolites is to aid in defense (Biomedicine 2012). It is possible that naphthoquinones are synthesized “to avoid the fast consumption of organic compounds by competitors before being absorbed by the pitcher cells” (Eilenberg *et al.* 2010). That is, *Nepenthes* may produce naphthoquinones as a way of preventing insects from decomposing due to fungal activity before the plant is able to digest them.

The Eilenberg *et al.* study in 2010 demonstrated that chitin-induced pitcher liquid exerts antifungal and fungicidal effects on the yeast strain *Candida albicans*. The article cited an *in vitro* experiment in which naphthoquinone derivatives inhibited *C. albicans* as effectively as Diflucan, the leading clinical yeast infection treatment (Tandon *et al.* 2009). Plumbagin is a naphthoquinone derivative that has been isolated and studied extensively due to its proven ability to inhibit and kill human pathogenic fungi (Eilenberg *et al.* 2010). The major problem with plumbagin is its high cytotoxicity and low therapeutic selectivity (Eilenberg *et al.* 2010). Droserone, an oxygenated derivative of plumbagin, exerted an antifungal effect on opportunistic fungal pathogens with a much lower level of toxicity. In addition, droserone demonstrated a different mechanism of action from that of mainstream antimycotics, like Diflucan. The majority of mainstream antimycotics prevent fungal growth by inhibiting fungal enzymes (Kayser *et al.* 2003). Droserone instead catabolizes, or breaks-down, fungal spores. This is advantageous considering the growing prevalence of antimycotic resistance (Kayser *et al.* 2003). In addition to inhibiting *C. albicans*, naphthoquinones demonstrated lethality to *Aspergillus*, a common household fungus which causes the life-threatening disease aspergillosis (Merck 2008).

Extracting secondary metabolites from plant cell cultures can often be a difficult and inefficient process (Eilenberg *et al.* 2010). When injected with chitin, *Nepenthes* pitchers produce moderate

amounts of medicinal compounds which require no additional purification (Eilenberg *et al.* 2010). In addition to serving as an antifungal agent, naphthoquinones derived from *Nepenthes* pitchers have the potential for a wide array of applications. These compounds are so versatile, they demonstrate antifungal, anti-cancer (Eilenberg *et al.* 2010; Sandur *et al.* 2006), anti-inflammatory (Lien *et al.* 1996), antimalarial (Biot *et al.* 2004), antiviral (Sacau *et al.* 2003), antiallergic (Lien *et al.* 1996), and antibacterial (Tandon *et al.* 2009) activities. Naphthoquinones may also serve as a totally “green” insect control because of their ability to inhibit insect ecdysis, or moulting, which is essential for insect survival (Eilenberg *et al.* 2010). Considering all of the potential applications in which *Nepenthes*’ chitin-induced pitcher liquid can be used, it is lucky that this tropical plant is able to produce exceptional yields of pure compounds, relatively simply.

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NEW CULTIVARS

Keywords: cultivar: *Dionaea muscipula* 'Orange Neat Trap', *Dionaea muscipula* 'Red Neat Trap', *Dionaea muscipula* 'Small Fast', *Dionaea muscipula* 'Viper Trap', *Pinguicula* 'Giant Green Violet', *Sarracenia* 'Narrow Throat'

Sarracenia 'Narrow Throat'

Submitted: 5 February 2013

Sarracenia 'Narrow Throat' is a *Sarracenia alata* hybrid of uncertain, but likely hybrid parents. The parents were purchased from Hwa-Jin Lee of carnivorous plant farm (Green Shark) in Hanam-si, Gyonggi-do, Republic of Korea. The crossbreeding of the parent plants was done by Jae-Hwan Lee in 2007. A few months later, about 100 seeds were separated. After cultivating for 2 years, one of the plants was selected and named 'Narrow Throat'.

The upper part of the pitcher tube is yellow and the lower part is green (Fig. 1). Red veins are spread evenly. The pitcher is about 40-60 cm long. The diameter depends on the height of the tube, but many are under 1 cm in diameter. The operculum is about 2 cm long and 2-3 cm wide and is entirely yellow with a red edge. The ends are bent around hazy red veins. The peristome is very thin and usually yellow, but sometimes a little reddish. The wing tends to be extremely wavy, becoming more severe in a hot climate. There are many cases when the width of the wing, about 2 cm, is longer than the diameter of the tube. The length of the wing is the same as the pitcher tube. Due to its small size, the pitchers have difficulty catching bugs. Digestive fluid also has a tendency to be wholly depleted at high temperatures, so it needs to grow almost through photosynthesis.



Figure 1: *Sarracenia* 'Narrow Throat' pitcher and plant.

The flower is mostly yellow or green. The bract is green with some brown. The petals are a bright yellow. It can bear seeds, but few in number.

The name ‘Narrow Throat’ reflects the difficulty of catching bugs and quickly drying digestive juices.

This cultivar should be reproduced asexually only.

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Pinguicula ‘Giant Green Violet’

Submitted: 5 February 2013

The parents of *Pinguicula* ‘Giant Green Violet’ are *Pinguicula zecheri* and a *Pinguicula agnata* hybrid. About 100 seeds were germinated in an interior greenhouse in winter 2007 by Jae-Hwan Lee. One of these plants showing great color and growth rate is ‘Giant Green Violet’. This cultivar usually grows much faster and healthier in wet soil than in dry soil.

The color of the flower is light violet and a violet vein on the petals has irregular patterns (Fig. 2). Sometimes petals can be fused or grow in an unusual, overlapped, or dislocated form. The petals are 2-3 cm long and 1.5-2 cm wide. The 10-cm flower stalk is brown and is usually stretched forward. The spur is 5-8 mm long, is entirely green, and grows in a curved shape

Table 1: Differences of <i>Pinguicula</i> ‘Giant Green Violet’ from similar cultivars.		
Cultivar	Leaf	Flower
‘Giant Green Violet’	Green with a light brown vein scattered irregularly; not wavy; tips grow downward.	Petals mostly wavy, sometimes fused; light violet with small random white patterns; violet veins with irregular patterns, darker toward center.
‘Gina’	Light green with a light green vein; wavy; slightly rolled up on margins.	Petals sometimes wavy; dark violet center, white middle, and light violet outer; light violet vein scattered irregularly; corolla mouth marked by yellow band.
‘Tina’	Green or light green with a light green vein; occasionally wavy.	Petals sometimes wavy; pale violet (mauve) with dark violet near center; violet veins darkest near center; white throat.

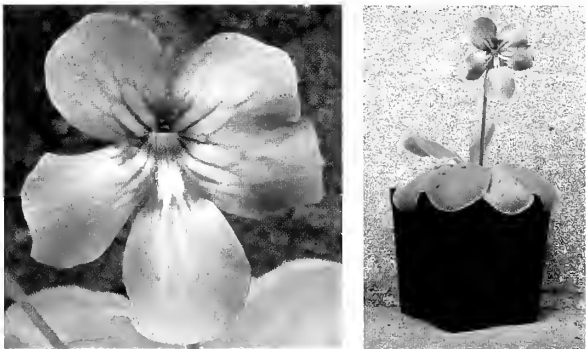


Figure 2: *Pinguicula* ‘Giant Green Violet’ flower and plant.

The mostly green leaves are 7-9 cm long and about 4 cm wide and do not become red in strong sunlight. On old leaves, a portion of the vein will have some brown color.

The name 'Giant Green Violet' was decided on 14 January 2013. The name 'Giant' suggests a large and vigorously growing plant, 'Green' is for the color of the leaves in the strong sunlight, and 'Violet' for the color of the flower.

This cultivar should be reproduced asexually only.

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Dionaea muscipula 'Red Neat Trap'

Submitted: 5 February 2013

Dionaea muscipula 'Red Neat Trap' was produced by breeder Jae-Hwan Lee in summer 2008 by crossbreeding a red form Venus flytrap with a form having many teeth.

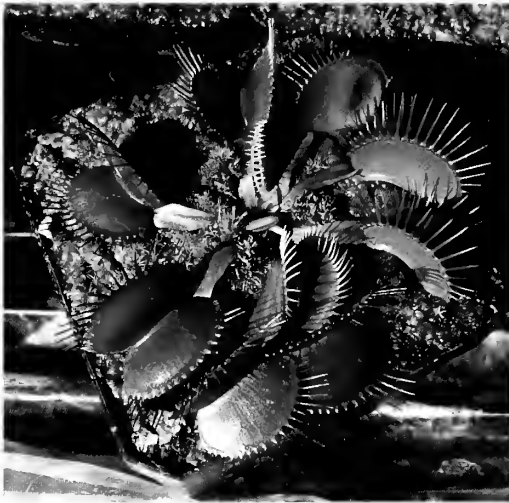


Figure 3. *Dionaea muscipula* 'Red Neat Trap'.

The inner side of the trap is always red or dark red, becoming darker toward the center (Fig. 3). There is some red color and a red line on outer side of the trap. The trap is 2-2.5 cm long and 1-1.5 cm wide. There are about 21 red teeth about 8 mm long. There are 3-6 black trigger hairs about 3 mm long with some additional small trigger hairs.

The petiole is about 2 cm long and 4 mm wide. The color is mixed green and red with a red line in the middle. The plant usually grows upright in a hot and humid location with strong sunlight.

The flower petals have some red, similar to the all red forms. There is one pistil and 15-16 stamens. It produces seeds normally. The stalk is about 15-25 cm long with 5-9 flowers. There are 5 calyxes.

The name 'Red Neat Trap' was decided on 12 January 2013 because it is entirely red and has a neat appearance.

This cultivar should be reproduced asexually only.

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Dionaea muscipula 'Viper Trap'

Submitted: 5 February 2013

Dionaea muscipula 'Viper Trap' was created in June 2007 from artificial crossbreeding between parents having a dentate form. The female parent was purchased in Yangjaedong Flowering Plant



Figure 4: *Dionaea muscipula* 'Viper Trap'. Note the unique trigger hairs.

At maturity, petioles, not including the trap, are about 7-10 cm long and 4-7 mm wide. The petioles are mostly green. During initial formation, they temporarily have a pink color. The plant form is an upright type.

The flower has one pistil and 13-17 stamens. The flower stalk is about 20-35 cm long and bears 4-11 flowers with 5 calyxes. It produces seeds normally.

The name 'Viper Trap' was decided on 12 January 2013 because the trap looks like the mouth of the viper and a trigger hair on the inner side of the trap looks like the teeth of the viper. The teeth on edge of the trap are similar to the scales of the viper.

This cultivar should be reproduced asexually only.

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Dionaea muscipula 'Orange Neat Trap'

Submitted: 5 February 2013

Dionaea muscipula 'Orange Neat Trap' was produced by breeder Jae-Hwan Lee in summer 2008 by crossbreeding an orange form Venus flytrap with a form having long teeth.

The inner side of the trap is mostly orange or sometimes pink (Fig. 5). Some observers may believe the color is red, but there is a clear difference between the color of 'Orange Neat Trap' and 'Red Neat Trap'. There is a faint orange line on outer side of the trap. The trap is about 2 cm long and 1 cm wide. There are an average of 16 red teeth that are about 1 cm long — somewhat longer than those of typical plants. The arrangement and length of the teeth is very regular. There are 3-4 reddish trigger hairs about 2 mm long.

Joint Market, Seoul by breeder Gun-Ju Kim, and after being cultivated for about a year, it was transferred to the breeder, Jae-Hwan Lee. The origin of the ancestor is likely the Korean Carnivorous Plant Institute. The male parent was cultivated by Jae-Hwan Lee. About a month after pollination, about 20 seeds were sown in the appropriate environment. Most of the seeds germinated and were grown for 2 years. One of the siblings is 'Viper Trap'.

Most of the inner side of the trap is a pink color (Fig. 4). The outer side of the trap is mostly green but often has a pink line. The trap is about 2.5-3.5 cm long and about 1.5-2 cm wide. The teeth are very short, 1-2 mm, and irregular. The edge can be pink, depending on the environment.

The trigger hairs have a unique appearance. The two at the top are large, about 5

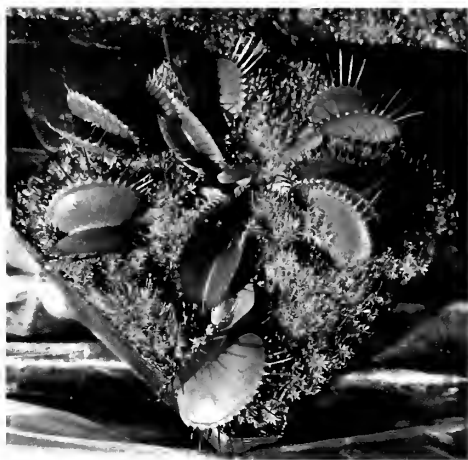


Figure 5: *Dionaea muscipula* 'Orange Neat Trap'.

The petiole is about 3 cm long and 5 mm wide. There is some red color and a red line in the middle of the petiole. The plant usually grows upright in a hot and humid location with strong sunlight.

The flower has one pistil and 13-15 stamens. It produces seeds normally. The stalk is about 15-25 cm long with 4-8 flowers. Occasionally 6 calyxes have been found.

The name 'Orange Neat Trap' was decided on 12 January 2013 because it has a neat appearance and usually has an orange color.

This cultivar should be reproduced asexually only.

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Dionaea muscipula 'Small Fast'

Submitted: 5 February 2013

In 2008, breeders Jae-Hwan Lee and Gun-Ju Kim selected normal Venus flytraps that had a rapid growth rate. These plants were crossbred by Jae-Hwan Lee. About a month later, about 200 seeds were collected and sowed. After a year of growth, one of the young plants showed a very rapid rate of growth. This plant was named 'Small Fast'. 'Small Fast' changes every day even during dormancy.

The inner side of the trap is red with differing tones (Fig. 6). The outer side is usually green, but it can be slightly red, depending on the environment. The trap is about 1.5 cm long and 6-8 mm wide. There is an average of 15 teeth per trap that are 3-5 mm long with an irregular array. When the teeth are dark red, their edge is white.

The trigger hairs are mostly red, white, or transparent. There are usually 8 trigger hairs about 2 mm long, 2 pair on the bottom and 2 pair on the top.

The length of the petiole is similar to the length of the trap and can be somewhat red, depending on the environment. The petiole is about 1-1.5 cm long and 3 mm wide.

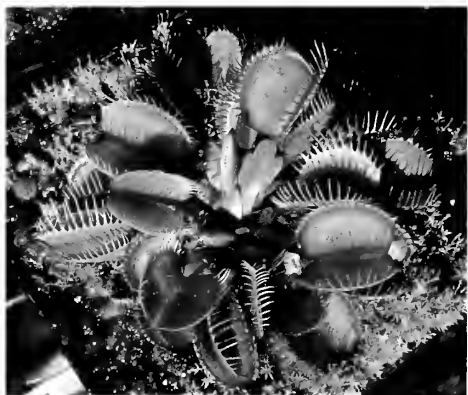


Figure 6: *Dionaea muscipula* 'Small Fast'. Note the white and transparent trigger hairs.

The flower has one pistil and 14-16 stamens. It can produce normal seeds. The flower stalk is 15-20 cm with 5-9 flowers. There are 5 calyxes.

The name 'Small Fast' was decided on 13 January 2013. 'Small' reflects it is half the size of normal Venus flytraps and 'Fast' reflects its faster rate of growth.

This cultivar should be reproduced asexually only.

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